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Abstract: Obesity is a widespread chronic inflammatory disorder characterized by an increased overall disease burden and significant association with periodontitis. The aim of this prospective clinical cohort study was to investigate the effect of obesity on orthodontic tooth movement. Fifty-five adolescent patients (27 males, 28 females) with a mean (SD) age of 15.1 (1.7) years and mean (SD) body mass index (BMI) of 30.2 (3.5) kg/m² in obese and 19.4 (2.2) kg/m² in normal-weight groups were followed from start of treatment to completion of tooth alignment with fixed orthodontic appliances. Primary outcome was time taken to complete tooth alignment, while secondary outcomes included rate of tooth movement and change in clinical parameters (plaque/gingival indices, unstimulated whole-mouth salivary flow rate, gingival crevicular fluid biomarkers). Data collection took place at baseline (start of treatment: appliance placement), 1 h and 1 wk following appliance placement, and completion of alignment. Results were analyzed by descriptive statistics followed by generalized estimating equation regression modeling. There were no significant differences between groups in time taken to achieve tooth alignment (mean [SD] 158.7 [75.3] d; $P = 0.486$). However, at 1 wk, initial tooth displacement was significantly increased in the obese group ($P < 0.001$), and after adjusting for confounders, obese patients had a significantly higher rate of tooth movement compared with normal-weight patients (+0.017 mm/d; 95% confidence interval, 0.008-0.025; $P < 0.001$) over the period of alignment. Explorative analyses indicated that levels of the adipokines leptin and resistin, the inflammatory marker myeloperoxidase (MPO), and the cytokine receptor for nuclear factor kappa-B ligand (RANKL) were significantly different between obese- and normal-weight patients and associated with observed rates of tooth movement. This represents the first prospective data demonstrating a different response in obese patients compared with normal-weight patients during early orthodontic treatment. These differences in the response of periodontal tissues to orthodontic force in the presence of obesity have potential short- and long-term clinical implications.

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Impact of obesity on orthodontic tooth movement in adolescents: a prospective clinical cohort study

Hayder F. Saloom ^{1,2,3}, Spyridon N. Papageorgiou ⁴, Guy H. Carpenter ², Martyn T. Cobourne ^{1*}

- (1) King's College London Dental Institute
Department of Orthodontics
London SE19RT, UK
- (2) King's College London Dental Institute
Department of Mucosal and Salivary Biology
London SE19RT, UK
- (3) Orthodontic Department
College of Dentistry
University of Baghdad, Iraq
- (4) University of Zurich
Clinic of Orthodontics and Pediatric Dentistry, Center of Dental Medicine
Zurich CH-8032, Switzerland

* Address for correspondence:
Professor Martyn Cobourne
Department of Craniofacial Development and Orthodontics,
King's College London Dental Institute,
Floor 27, Guy's Hospital,
London SE1 9RT, UK
Tel: +44 (0) 2071888028
Fax: +44 (0) 2071884415
Email: martyn.cobourne@kcl.ac.uk.co.uk

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Abstract

Obesity is a widespread chronic inflammatory disorder characterized by an increased overall disease burden and significant association with periodontitis. The aim of this prospective clinical cohort study was to investigate the effect of obesity on orthodontic tooth movement. Fifty-five patients (27 males, 28 females) mean age 15.1 (SD, 1.7) years and mean body mass index 30.2 (3.5) in obese and 19.4 (2.2) kg/m² in normal-weight groups were followed from start-of-treatment to completion of tooth alignment with fixed-appliances. Primary-outcome was time taken to complete tooth alignment, whilst secondary-outcomes included rate of tooth movement and change in clinical parameters (plaque/gingival indices, unstimulated whole mouth salivary flow rate and gingival crevicular fluid biomarkers). Data collection took place at baseline (start-of-treatment at appliance-placement); at 1-hour and 1-week following appliance-placement; and completion of alignment. Results were analyzed by descriptive statistics followed by generalized estimating equation regression modelling. Obese patients needed slightly less time to complete tooth alignment compared to normal-weight, but this was non-significant for both crude and adjusted analyses. However, after adjusting for confounders obese patients had a significantly higher rate of tooth movement compared to normal-weight (+0.017 mm/day; 95 CI: 0.008,0.025; P<0.001) over the period of alignment. Explorative analyses indicated that levels of the adipokines leptin and resistin, the inflammatory-marker myeloperoxidase and the cytokine receptor for nuclear factor kappa-B ligand (RANKL) were significantly different between obese and normal-weight patients and associated with the observed rates of tooth movement. This represents the first prospective data demonstrating a different response in obese patients compared to normal-weight during orthodontic tooth movement. These differences in the response of periodontal tissues to orthodontic force in the presence of obesity have potential short and long-term clinical implications.

Introduction

Obesity levels have been rising amongst children and adults in Western societies over the last few decades (Ng et al. 2014). This represents a major healthcare challenge because of the known associations between raised body mass index (BMI) and multiple chronic diseases, including insulin-resistant diabetes, cardiovascular disease and cancer (Deng et al. 2016). It is recognized that obesity represents a state of chronic subclinical inflammation mediated through the presence of excess adipose tissue (Hotamisligil 2006). Adipocytes are the predominant cellular component of white adipose and produce a host of metabolically-active proteins or adipokines, which influence metabolic function and inflammatory responses (Ouchi et al. 2011). These include the pro-inflammatory adipokines leptin (Zhang et al. 1994) and resistin (Steppan et al. 2001) and the anti-inflammatory adiponectin (Scherer et al. 1995).

Adipose tissue can influence the intensity and resolution of inflammatory responses in multiple tissues (Issa and Griffin 2012; Pierpont et al. 2014). Indeed, there is evidence of an increased risk of chronic periodontitis (Keller et al. 2015; Suvan et al. 2011) and variation in inflammatory and metabolic markers in obese subjects affected by periodontal disease when compared to normal-weight (Papageorgiou et al. 2015a). The systemic induction of inflammatory markers may provide a link between obesity and periodontitis, with some current focus on C-reactive protein (CRP) as an important potential mediator (Pradeep et al. 2012). Orthodontic tooth movement takes place as a direct consequence of connective tissue remodeling within the periodontium and alveolar bone, which occurs through a localized inflammatory response induced by external force (Meikle 2006). This triggers the release of essential biochemical mediators, which are often detectable within gingival crevicular fluid (GCF) (Kapoor et al. 2014). In particular, the tissue-modulating factors receptor for nuclear factor kappa-B ligand (RANKL), matrix metalloproteinases (MMPs) and tissue-inhibitors of MMPs (TIMPs) (Grant et al. 2013). Obesity is also known to influence systemic bone metabolism through complex mechanical, hormonal, and inflammatory interactions (Lopez-Gomez et al. 2016) with associations between obesity and reduced bone remodeling (Ivaska et al. 2016) and increased bone mineral density (Salamat et al. 2016). Although there is little data relating obesity to changes in alveolar bone composition within the healthy periodontium,

longitudinal data has shown a significant association with increased rates of tooth eruption (Must et al. 2012).

The potential implications of adolescent obesity for orthodontic treatment have been highlighted (Neeley and Gonzales 2007) with increased BMI a risk factor for less cooperation and longer treatment duration with fixed appliances (von Bremen et al. 2016). However, despite known associations between raised BMI and chronic inflammatory changes within the periodontium, there have been no prospective investigations of orthodontic tooth movement in obese patients. The aim of this study was to investigate the effect of obesity on orthodontic tooth movement during routine treatment with fixed-appliances. Specifically, time-taken to complete tooth alignment and variation in clinical parameters, including GCF biomarkers.

Materials and Methods

Study design

This prospective cohort study compared the effects of obesity on tooth alignment with fixed-appliances. Ethical approval was obtained from the United Kingdom National Research Ethics Service (UK NRES) (14/LO/0769). Written-informed consent was received from all parents, guardians and children. We report and present data according to STROBE (von Elm et al. 2008).

Setting

Participants fulfilling the inclusion criteria were recruited consecutively from orthodontic treatment clinics at King's College London Dental Institute (Guy's Hospital) UK between January 2015-January 2016. Follow-up occurred to June 2016 and covered appliance placement to completion of alignment.

Participants

Inclusion criteria included: (1) fixed-appliance treatment; (2) 12-18 years-old at treatment-start; (3) no medical contraindications or regular medication (including antibiotic-therapy) in previous six-months; (4) non-smokers; (5) permanent dentition; (6) mandibular arch incisor irregularity

index of 4-12 mm; (7) normal-weight (BMI-centile 2-91) and obese (BMI-centile >98) classification. Those classified as underweight (BMI-centile <2) and overweight (BMI-centile 91-98) were excluded, respectively.

Subject body weight was measured to the nearest 0.1 kilogram (kg) using a calibrated scale and height measured to the nearest centimeter (cm) using a wall-mounted rule. BMI was calculated as mass (kg) divided by height in meters-squared (kg/m^2). United Kingdom Royal College of Pediatrics and Child Health World Health Organization growth-charts were used to calculate and classify BMI-centile in relation to age and sex. All measurements were taken by a single-trained operator (HFS) using the same equipment.

Variables

Tooth alignment was calculated from scanned (3Shape R700, Copenhagen, Denmark) stone dental casts using an irregularity-index (Little 1975).

Unstimulated whole mouth salivary flow rate (uWMS) was calculated as millilitre (mL) per minute from saliva obtained from relaxed patients spitting into a plastic tube for five-minutes. Periodontal health was measured clinically using established-validated plaque and gingival indices (Loe and Silness 1963; Silness and Loe 1964). GCF was collected from the distal side of the lower six anterior teeth and pooled. Following isolation, teeth were gently dried using an air-syringe and filter-paper strips (Periopaper, OraFlow Inc. New York, USA) placed 1 mm into the gingival crevice for 30 seconds. If there was any contamination of the strip with saliva or blood it was discarded. The volume of collected fluid in the strip was measured directly using a Periotron 8000 electronic micro-moisture meter (OraFlow Inc. New York, USA) with readings converted to an actual volume by reference to the standard curve and flow-rate calculated (per minute). GCF was retrieved from filter strips with the addition of 20 μl PBS and centrifugation for 5 minutes at 9200g. Samples were stored at -80 °C for subsequent analysis. GCF was analyzed by Luminex bead-based multiplex assay using a commercially available kit (R&D systems, Abingdon, UK) for detection (pg/mL) of the adipokines adiponectin, leptin and resistin; the inflammatory mediators myeloperoxidase (MPO) and CRP, and the tissue-remodeling biomarkers MMP8, MMP9, TIMP1, MMP8/TIMP1,

MMP9/TIMP1 and RANKL (Appendix Table 1).

Fixed-appliances and bonding-method were standardized between groups (3M Victory-APC 0.022-inch brackets, MBT prescription, 3M-Unitek, Monrovia, USA). After bracket-bonding, a 0.014-inch nickel-titanium archwire was tied in and ligated using conventional elastomerics. The archwire was cut distal to the first molar teeth and not cinched. No bite planes, auxiliary-arches, inter-maxillary elastics, headgears or temporary anchorage devices were used during the investigation. All appliances were placed by postgraduate orthodontic trainees under direct supervision of a consultant orthodontist.

Sample collection took place during routine appointments between the hours of 9.30 am to 3.30 pm at baseline (start-of-treatment at appliance placement) (irregularity index, uWMS, plaque and gingival indices, GCF flow-rate, biomarker-analysis); 1-hour following appliance placement (GCF flow-rate, biomarker-analysis), 1-week following appliance placement (irregularity index, uWMS, plaque and gingival indices, GCF flow-rate, biomarker-analysis); and at completion of tooth alignment (0.019 x 0.025-inch stainless-steel archwire) (irregularity index, uWMS, plaque and gingival indices, GCF flow-rate, biomarker-analysis). This was a pragmatic study undertaken in a clinical department. Patients were seen at approximate 6-week intervals for appliance adjustment and archwire progression took place as deemed clinically appropriate by treating clinicians. Patient-flow through the study is shown in Appendix Figure 1.

Primary outcome was time to achieve tooth alignment in the lower arch. Secondary outcomes included rate of tooth movement, changes in clinical parameters and GCF biomarkers during treatment. There were no changes to outcomes following study commencement.

Sample size

Sample size was based upon previous randomized prospective data on time to completion of alignment with fixed-appliances, which found a mean time-to-alignment of 200.7 days with standard deviation (SD) 73.6 days in the presence of 8.9 mm incisor irregularity (Woodhouse et al. 2015). A total of 50 patients were required to detect with an unpaired t-test a

hypothesized 30% reduction (Schulz and Grimes 2005) in alignment time with a common SD across groups to yield 80% power ($P=0.05$). Five additional patients were recruited to account for possible drop-outs.

Statistical methods

Descriptive statistics were calculated, after checking for normal distribution. All biomarker data were Log_{10} transformed for normalization. Initial crude differences in baseline and outcome data were calculated with independent t-tests, chi-square tests or Mann-Whitney tests, where needed.

The effect of obesity was investigated using univariable (crude) and multivariable generalized estimation equation regression models with robust standard errors to take into account correlation between repeated measurements for each patient through the follow-up period (baseline, 1 hour, 1 week, and completion of alignment), adjusted for the confounding effect of baseline data (sex, age, baseline-irregularity). Results are reported as unstandardized coefficients or Odds Ratios (ORs) for continuous and binary outcomes, respectively. The effect modification of obesity on the progress of tooth alignment was tested by introducing interaction terms, which were ultimately dropped from the model if not significant. Analysis of residuals was conducted to confirm the regression assumptions. As patients within the study had initial irregularity ranging from 4-12 mm, sensitivity analyses were conducted to include only those with severe (≥ 7 mm) or moderate (4-7 mm) baseline-irregularity. All analyses were carried out using Stata 12.0 (Statacorp College Station, Texas, USA). A 2-tailed P-value of 0.05 was considered statistically significant with a 95% Confidence Interval (CI) for all tests.

All primary data was coded so that the outcome assessor (HFS) and statistician (SNP) were blinded to subject classification. Data-coding was broken following analysis and no blinding breaches were identified. To examine measurement reliability and agreement, 36-pairs of models from baseline and 1 week were selected and re-measured after 2-weeks. The Concordance Correlation Coefficient (CCC) (Lin 1989) and Bland-Altman method (Bland and Altman 1986) were used to test intra-examiner reliability and agreement.

Results

Participants

This study included 55 patients (27 male, 28 female) mean age 15.1 (SD 1.7) years and mean irregularity index 7.6 (SD 2.4; 95% CI 6.9-8.2) mm. Mean BMI of the cohort was 24.7 (SD 6.2) kg/m². From the original 55 patients recruited, 7 were excluded at 1 week due to missed appointments, but all 55 were included at completion of alignment. Missingness at 1 week was judged as random (Appendix Table 2). The reliability and agreement of repeated measurements was excellent (CCC: 0.99 with 95% CI: 0.98 to 0.99; average difference 0.06 with 95% limits of agreement: -0.68 to 0.79). Table I shows demographics and GCF parameters for the two cohorts at baseline. Mean BMI was 19.4 (2.2) in the normal-weight group and 30.2 (3.5) kg/m² in the obese group. Apart from BMI, there were no statistically significant differences in demographics among groups at baseline; however, the obese group did have 1.2 mm more irregularity (P=0.061). In contrast, significant differences were present between normal-weight and obese groups for a number of GCF biomarkers at baseline (Table I; P<0.05) including increased GCF flow-rate, increased leptin, resistin, MPO, MMP8, TIMP1 and RANKL, and reduced MMP9/TIMP1 levels in obese patients.

Primary outcome

The results of both crude and adjusted regression analyses indicated a small difference in time required to achieve tooth alignment between obese and normal-weight patients. Overall, obese patients needed a mean 23.0 days less than normal-weight to reach final alignment, but this was not statistically significant (Table II, P>0.05).

Secondary outcomes

Rates of tooth movement were significantly increased in obese patients compared to normal-weight during the first week (P<0.001) and overall (P=0.05) (Table III), which was confirmed by regression modelling of the whole alignment process (Table II; P<0.001). After taking into account all confounders in the adjusted analysis, obese patients were associated with a

significantly increased rate of tooth movement compared to normal-weight (0.017 mm/day; 95% CI: 0.008, 0.025 mm/day) (Figure 1). Additionally, a significant association was found between rate of tooth movement and initial irregularity (0.007 mm/day increase per mm irregularity). Sensitivity analyses for patients with either severe or moderate irregularity were consistent in direction with the main analysis (Appendix Tables 3 and 4, respectively) with an expected loss of power due to the division of the study sample, and a higher difference in alignment rate between obese and normal-weight patients in the severe irregularity group.

uWMS increased during treatment, whilst plaque and gingival indices deteriorated significantly (Appendix Table 5), but there were no differences between groups for either of these parameters. GCF flow-rate increased during orthodontic treatment for both groups, but significantly more in obese patients.

In order to further understand the biochemical basis of observed differences in rates of tooth movement, explorative regression analyses were undertaken (Table IV). GCF levels of leptin, resistin, MPO, MMP8, TIMP1, MMP9/TIMP1 and RANKL were significantly different between obese and normal-weight patients at baseline and during subsequent assays (Appendix Table 5). When a possible inter-relation between these biomarkers and rate of tooth movement was investigated, it was found that leptin, resistin, MPO and RANKL were significantly associated with the amount of tooth movement for each patient (Appendix Table 6). Therefore, from an epidemiological basis, these biomarkers are the best candidates to explain the clinical performance difference between obese and normal-weight patients during orthodontic tooth alignment with fixed appliances.

Discussion

This prospective study followed a cohort of obese and normal-weight adolescent patients during the alignment phase of fixed-appliance orthodontic treatment. Obese patients needed less time to achieve alignment but this was not statistically significant. However, obese patients did show significantly increased rates of tooth movement during the whole observation period. This apparent discrepancy might be explained by the slightly (albeit statistically non-significant) increased baseline irregularity present in the obese group, or

between-group variation in attendance during routine appointments. Evidence exists from a similar experimental model that initial alignment can increase by 0.01 mm per day whilst overall alignment increases by 0.004 mm per day for every millimeter of initial irregularity (Woodhouse et al. 2015). However, significant differences were also found in the GCF biochemical profile between obese and normal-weight patients and to our knowledge, this represents the first prospective data to suggest that obese patients may respond differently to those with normal-weight during routine orthodontic treatment.

Appliance variation has little or no effect on rate of orthodontic tooth movement (Scott et al. 2008; Woodhouse et al. 2015). Interestingly, we found that obesity does influence tooth movement, as obese patients had increased rates when compared to normal-weight. Statistical modelling of alignment rate and its change through time (see Table II) demonstrated that obesity and initial irregularity at each phase explained part of the variation seen in alignment. Given the absence of a significant interaction between obesity and time, the difference in alignment rate between obese and non-obese patients was consistently present through the alignment process and independent of confounders.

Importantly, the groups in this investigation were not different in terms of baseline demographics, including plaque/gingival indices and irregularity, with BMI representing the only significant difference. However, a number of differences existed in baseline GCF parameters between groups, including GCF flow-rate and levels of several biomarkers. The pro-inflammatory adipokines leptin and resistin were both elevated in GCF of obese patients (Suresh et al. 2016), suggestive of a baseline pro-inflammatory state within the periodontium of these individuals. It is also consistent with the significantly increased levels of MPO, an established marker for inflammation in the GCF (Marcaccini et al. 2010; Navarro-Palacios et al. 2014). Interestingly, the levels of several biochemical mediators of tissue remodelling were also increased at baseline in the obese group, including MMP8, TIMP1, MMP9/TIMP1 and RANKL, providing evidence of an altered inflammatory biochemical profile in the GCF of obese patients.

Amongst the GCF biomarkers assayed, leptin, resistin, MPO, and RANKL most predictably accounted for the observed differences in rate of tooth movement. The levels of

these biomarkers differed significantly between obese and normal-weight patients both before and during treatment; whilst at the same time, being significantly associated with the amount of tooth movement observed. Previous studies have reported that orthodontic tooth movement is followed by a decrease in GCF leptin (Dilsiz et al. 2010) and an increase in both MPO (Marcaccini et al. 2010; Navarro-Palacios et al. 2014) and RANKL (Grant et al. 2013). Resistin, like leptin, is upregulated in inflamed gingival tissue as compared to healthy (Suresh et al. 2016), but the relationship between GCF resistin and orthodontic tooth movement has not previously been investigated. Variation in the levels of pro-inflammatory adipokines have been identified in the GCF of obese and normal-weight individuals with periodontal disease (Duzagac et al. 2016; Goncalves et al. 2015; Suresh et al. 2016; Zimmermann et al. 2013), but data relating to adipokines during orthodontic tooth movement is sparse (Dilsiz et al. 2010).

The strengths of the present study include its prospective design (Papageorgiou et al. 2015b), baseline comparability between experimental groups, absence of drop-outs at completion and use of measurement-blinding. Moreover, obesity was defined according to widely-accepted and reliable international measures. Collectively, this means that the respective risk for selection, attrition and detection bias is low. The study sample was based on a conservative *a priori* power calculation, and planned drop-outs did not occur. However, some potential limitations include the fact that height and weight measurements were only taken at baseline and adiposity is not necessarily a static measure. Indeed, in an adolescent population underlying growth might have influenced BMI during the course of the investigation, although with a mean observation of 158 days and mean patient age of 15.1 years, this effect may have been negligible. In addition, only BMI was used to classify adiposity, which can limit the identification of overweight and could have been reduced by adding estimates of adiposity (fat mass index) and fat distribution (waist-to-height ratio) (Bibiloni Mdel et al. 2013). Moreover, in a cohort undergoing routine orthodontic treatment with fixed appliances it is not practical to see each patient at exactly the same time-point for each adjustment, or identify the absolute first time-point that alignment is complete for every patient. For these reasons, the increased rates of tooth movement identified in the obese group may not have resulted in a clinically significant reduction in time to final alignment. In addition, whilst observed differences in rates

of tooth movement are tangible effects with obvious potential clinical relevance, the underlying biological mechanisms are likely to be complex. The measured differences in GCF biomarkers may be associated with the inter-relationship between obesity and tooth movement, but this study provides no conclusive evidence. Further investigation will be required to elucidate the precise role of each biomarker in mediating tooth movement. However, this investigation provides evidence that informs clinical practice both in orthodontics and wider-healthcare. The results are applicable to obese and normal-weight adolescent patients, although it should be remembered that adipose tissue can behave differently according to age group in other body systems (Palmer and Kirkland 2016). A pro-inflammatory obese state can influence orthodontic tooth movement, with significant associations between levels of specific biomarkers within the GCF of obese patients. These results highlight potential implications for orthodontic treatment in obese subjects and one area for future research would be a comparison of post-orthodontic stability.

This prospective clinical study investigated tooth alignment in obese and normal-weight patients undergoing fixed-appliance orthodontic treatment. Obese patients needed less time to achieve tooth alignment compared to normal-weight, but this was non-significant. After adjusting for confounders, rate of orthodontic tooth movement was significantly higher in obese patients compared to normal-weight. Explorative analyses indicated GCF-levels of leptin, resistin, MPO and RANKL were significantly different between obese and normal-weight patients and associated with observed rates of tooth movement.

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Figure legends

Figure 1 Box plots of measured values in normal-weight and obese patients for primary (time to completion of alignment in days; left panel) and secondary (tooth alignment rate from start of treatment to completion of alignment in mm/day; right panel). Plotted boxes with horizontal lines indicate interquartile ranges with medians. Vertical whiskers and points indicate upper and lower adjacent values and outliers.

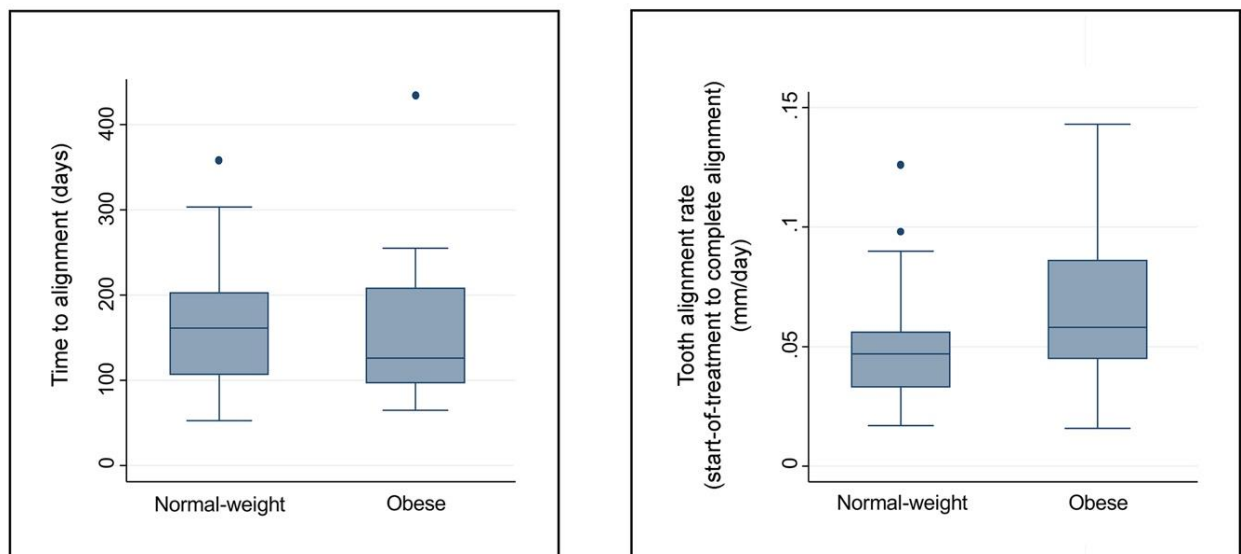


Table I Demographics and GCF parameters of patients at baseline

| | Overall | Normal-weight | Obese | P value |
|--|---------------|---------------|---------------|------------------------------|
| Demographics | | | | |
| Patients (n) | 55 | 28 | 27 | |
| Male / female (n) | 27/28 | 15/13 | 12/15 | 0.498* |
| Age (years) | 15.1 (1.7) | 15.1 (1.6) | 15.1 (1.9) | 0.991 [#] |
| Caucasian – n(%) | 29 (53) | 17 (61) | 12 (44) | 0.516* |
| Asian – n(%) | 4 (7) | 2 (7) | 2 (7) | |
| African – n(%) | 5 (9) | 1 (4) | 4 (15) | |
| Mixed – n(%) | 8 (15) | 3 (11) | 5 (19) | |
| Other – n(%) | 9 (16) | 5 (18) | 4 (15) | |
| BMI (kg/m ²) | 24.7 (6.2) | 19.4 (2.2) | 30.2 (3.5) | <0.001[#] |
| uWMS (mL/min) | 0.61 (0.32) | 0.64 (0.34) | 0.58 (0.29) | 0.480 [#] |
| Plaque index | 0.56 (0.32) | 0.57 (0.32) | 0.54 (0.31) | 0.745 [#] |
| Gingival index | 0.74 (0.39) | 0.74 (0.40) | 0.73 (0.38) | 0.934 [#] |
| Irregularity index (mm) | 7.6 (2.4) | 7.0 (2.3) | 8.2 (2.4) | 0.061 [#] |
| Severe irregularity – n (%) | 31 (56) | 13 (46) | 18 (67) | 0.130* |
| Tooth extraction – n (%) | 8 (15) | 4 (14) | 4 (15) | 0.956* |
| GCF biomarkers (Log ₁₀ transformed) | | | | |
| GCF flow-rate (μL/min) | -0.29 (0.14) | -0.33 (0.12) | -0.25 (0.12) | 0.011[£] |
| | | | | |
| Adiponectin | 6.60 (0.34) | 6.55 (0.42) | 6.66 (0.23) | 0.237 [#] |
| Leptin [‡] | 13.91 (22.64) | 6.40 (14.65) | 19.15 (24.45) | 0.031[£] |
| Resistin | 5.61 (0.56) | 5.30 (0.55) | 5.92 (0.36) | <0.001[#] |
| | | | | |
| MPO | 5.05 (0.85) | 4.44 (0.64) | 5.69 (0.52) | <0.001[#] |
| CRP | 2.50 (0.51) | 2.47 (0.27) | 2.65 (0.87) | 0.827 [#] |
| MMP8 | 6.32 (0.64) | 6.01 (0.48) | 6.64 (0.64) | <0.001[#] |
| MMP9 | 6.23 (0.30) | 6.18 (0.59) | 6.27 (0.15) | 0.245 [#] |
| TIMP1 | 5.00 (0.48) | 4.72 (0.38) | 5.28 (0.40) | <0.001[#] |
| MMP8/TIMP1 | 0.96 (0.64) | 0.93 (0.62) | 0.99 (0.68) | 0.699 [#] |
| MMP9/TIMP1 | 0.54 (0.54) | 0.75 (0.54) | 0.32 (0.47) | 0.002[#] |
| RANKL | 3.54 (0.33) | 3.39 (0.25) | 3.65 (0.27) | <0.001[£] |

For demographics: values are mean (SD) unless otherwise indicated.

For GCF parameters: values are pg/mL unless otherwise indicated

BMI, body mass index; uWMS, unstimulated whole mouth salivary flow rate; GCF, gingival crevicular fluid; MPO, myeloperoxidase; CRP, C-reactive protein; MMP8, matrix metalloproteinase-8; MMP9, matrix metalloproteinase-9; TIMP1, tissue inhibitor of metalloproteinase 1; RANKL, receptor activator of nuclear factor kappa-B ligand.

* from chi-square test.

[#] from independent t-test.

[£] from Mann-Whitney test.

Log₁₀ transformation improved the skewness of the data, but the Shapiro-Wilk test indicated that transformed data were still not normally distributed. Therefore, the median (interquartile range) is presented instead of mean (SD) and the Mann-Whitney test is used on the transformed data instead of the unpaired t-test.

[‡] Square root transformation used instead of Log₁₀, as several null values were included. Therefore, the median (interquartile range) is presented instead of mean (SD) and the Mann-Whitney test is used on the transformed data instead of the unpaired t-test.

Significant results are indicated in bold

Table II Regression analysis on primary (time to achieve tooth alignment in days) and secondary (rate of orthodontic tooth movement in mm/day) outcomes

| Primary outcome (time to completion of alignment) | | Crude model | | | Adjusted model | | |
|---|--------------------|--------------------|---------------|--------|-----------------------|---------------|------------------|
| Factor | | b | 95% CI | P | b | 95% CI | P |
| BMI group | Obese | -14.3 | -54.3,25.7 | 0.483 | -23.0 | -66.1,20.1 | 0.295 |
| | Control | <i>Ref</i> | | | | | |
| Age | Per year | <i>NT</i> | | | -3.2 | -15.6,9.1 | 0.608 |
| Gender | Male | <i>NT</i> | | | -6.6 | -48.4,35.2 | 0.756 |
| | Female | <i>NT</i> | | | | | |
| Ethnicity | | <i>NT</i> | | | -0.2 | -13.4,13.0 | 0.974 |
| Extraction | Yes | <i>NT</i> | | | -15.3 | -76.0,45.5 | 0.623 |
| | No | <i>NT</i> | | | | | |
| Baseline irregularity | Per mm | <i>NT</i> | | | 6.9 | -2.5,16.3 | 0.151 |
| Secondary outcome (alignment rate: baseline-to completion of alignment) | | Crude model | | | Adjusted model | | |
| Factor | | b | 95% CI | P | b | 95% CI | P |
| BMI group | Obese | 0.023 | 0.011,0.035 | <0.001 | 0.017 | 0.008,0.025 | <0.001 |
| | Control | <i>Ref</i> | | | <i>Ref</i> | | |
| Time | 1 week | <i>Ref</i> | | | <i>Ref</i> | | |
| | Complete alignment | -0.025 | -0.036,-0.015 | <0.001 | -0.021 | -0.032,-0.010 | <0.001 |
| Age | Per year | <i>NT</i> | | | 0.001 | -0.002,0.003 | 0.609 |
| Gender | Male | <i>NT</i> | | | 0.001 | -0.008,0.011 | 0.768 |
| | Female | <i>NT</i> | | | <i>Ref</i> | | |
| Ethnicity | | <i>NT</i> | | | -0.001 | -0.003,0.002 | 0.643 |
| Irregularity at each phase start | Per mm | <i>NT</i> | | | 0.007 | 0.005,0.009 | <0.001 |
| Extraction | Yes | <i>NT</i> | | | -0.004 | -0.014,0.006 | 0.415 |
| | No | <i>NT</i> | | | <i>Ref</i> | | |

b, unstandardized regression coefficient; CI confidence interval; BMI, body mass index; Ref, reference; NT, not tested. *Interaction of obesity with time found to be non-significant (P=0.112) and was dropped from the model. Significant results are indicated in bold

Table III Rate of tooth movement (mm/day) during the study period

| Outcome | Overall (n=55) | Normal-weight (n=28) | Obese (n=27) | P value |
|---|---------------------------|---------------------------------|-------------------------|-------------------|
| Time to completion of alignment (d) – mean (SD) | 158.7 (75.3) | 165.8 (72.5) | 151.4 (78.7) | 0.486 |
| Tooth alignment rate: baseline to completion of alignment (mm/d) – mean (SD) | 0.057 (0.029) | 0.050 (0.025) | 0.065 (0.031) | 0.050* |
| Tooth alignment rate: baseline to week 1 (mm/d) – mean (SD) [†] | 0.081 (0.031) | 0.065 (0.025) | 0.097 (0.028) | <0.001* |
| Tooth alignment rate: week 1 to completion of alignment (mm/d) – mean (SD) [†] | 0.056 (0.031) | 0.049 (0.027) | 0.063 (0.033) | 0.119* |

d, days; SD, standard deviation; CI, confidence interval.

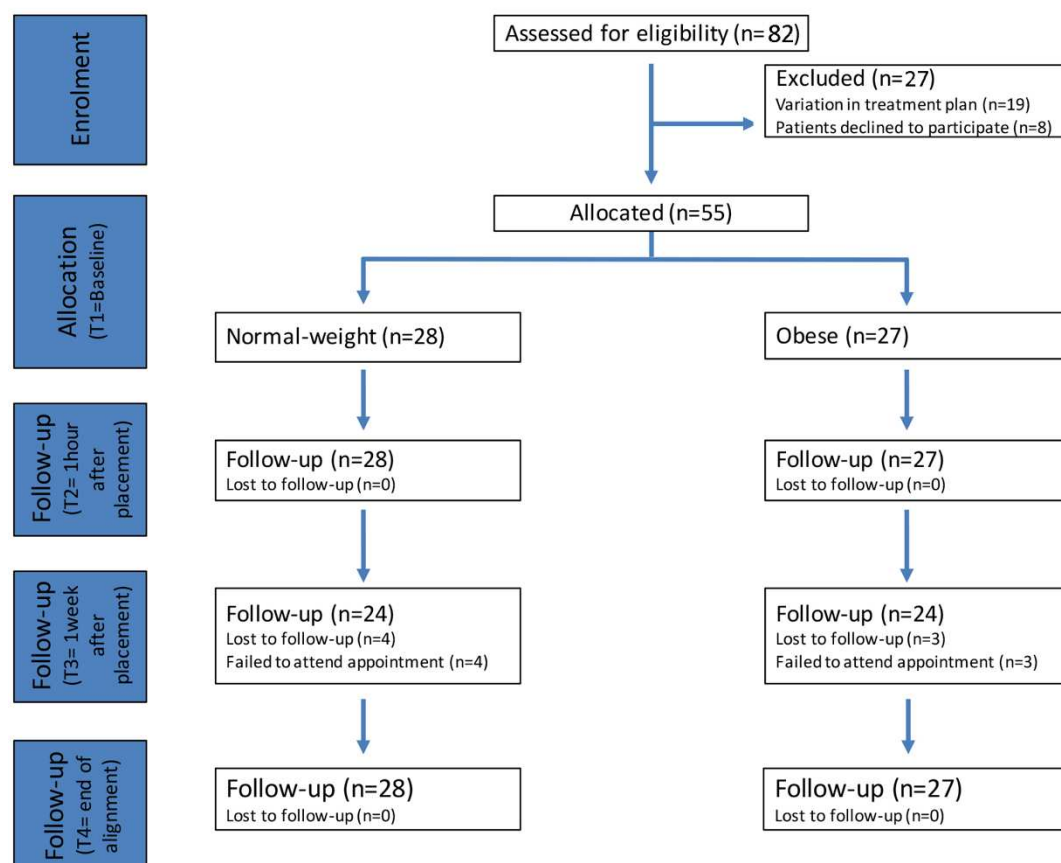
*from independent t-test.

[†]Due to 7 patient drop-outs at 1 week, 48/55 patients (24 obese and 24 control patients) are included in these two measurements. The measurement of time to completion of alignment and alignment rate: baseline to completion of alignment pertain to the whole sample of 55 patients.

Table IV Summary of exploratory analyses on the secondary outcome: GCF biomarker levels

| | | Different in obese and control at baseline (Table I) | Different in obese and control during treatment (Appendix Table 5) | Associated with alignment rate (Appendix Table 6) |
|------------------|----------------|--|---|--|
| Clinical indices | | | | |
| | Plaque index | No | No | - |
| | Gingival index | No | No | - |
| | uWMS | No | No | - |
| | GCF volume | Yes | Yes | No |
| GCF biomarkers | | | | |
| | Adiponectin | No | No | - |
| | Leptin | Yes | Yes | Yes |
| | Resistin | Yes | Yes | Yes |
| | MPO | Yes | Yes | Yes |
| | CRP | No | No | - |
| | MMP8 | Yes | Yes | No |
| | MMP9 | No | No | - |
| | TIMP1 | Yes | Yes | No |
| | MMP8/TIMP1 | No | No | - |
| | MMP9/TIMP1 | Yes | Yes | No |
| | RANKL | Yes | Yes | Yes |

Appendix Figure 1 Patient flow through the investigation



Appendix Table 1 Limits of detection and coefficients of variation for GCF biomarker luminex analysis

| Biomarker * | Lower limit of detection (pg/mL) | Upper limit of detection (pg/mL) | Coefficient of variation (%) |
|--------------------|---|---|-------------------------------------|
| Adiponectin | 193.74 | 432390.10 | 2.4 |
| Leptin | 55.15 | 111251.22 | 3.0 |
| Resistin | 4.83 | 9709.28 | 1.8 |
| MMP8 | 25.45 | 53522.68 | 2.3 |
| MMP9 | 20.03 | 41870.79 | 2.8 |
| TIMP1 | 7.91 | 17479.14 | 2.1 |
| MPO | 16.31 | 34627.04 | 2.8 |
| CRP | 17.29 | 38785.30 | 2.4 |
| RANKL | 5.14 | 10509.81 | 2.3 |

* all manufactured by R+D Systems

Appendix Table 2 Demographics of the initially-recruited sample at baseline and the sample after patient drop-outs at 1 week

| | Overall | Control | Obese | P value |
|---|----------------|----------------|--------------|----------------|
| Recruited sample (data for baseline, completion of alignment) | | | | |
| Patients | 55 | 28 | 27 | |
| Male - n (%) | 27 (49%) | 15 (54%) | 12 (44%) | 0.498* |
| Age - mean (SD) | 15.1 (1.7) | 15.1 (1.6) | 15.1 (1.9) | 0.991# |
| Irregularity - mean (SD) | 7.6 (2.4) | 7.0 (2.3) | 8.2 (2.4) | 0.061# |
| BMI - mean (SD) | 24.7 (6.2) | 19.4 (2.2) | 30.2 (3.5) | <0.001# |
| | | | | |
| Sample with drop-outs (data for 1 week) | | | | |
| Patients | 48 | 24 | 24 | |
| Male - n (%) | 24 (50%) | 13 (54%) | 11 (46%) | 0.564* |
| Age - mean (SD) | 15.3 (1.7) | 15.2 (1.6) | 15.3 (1.8) | 0.737# |
| Irregularity - mean (SD) | 7.7 (2.4) | 7.2 (2.3) | 8.2 (2.5) | 0.156# |
| BMI - mean (SD) | 24.7 (6.2) | 19.3 (2.2) | 30.2 (3.6) | <0.001# |

SD, standard deviation; BMI, body mass index.

Appendix Table 3 Sensitivity analysis: regression analysis on the primary (time to completion of alignment in days) and secondary outcome (rate of tooth movement in mm/day) for the category of patients with severe initial crowding (≥ 7 mm; n=31)

| | | Factor | | Crude model | | | Adjusted model | | |
|---|------------------------------------|----------------------------------|----------|-------------|---------------|--------|----------------|----------------|--------|
| | | | | b | 95% CI | P | b | 95% CI | P |
| Sensitivity analysis: initial crowding ≥ 7 mm (n=31) | Primary outcome: time to alignment | BMI group | Obese | -16.0 | -67.9,35.9 | 0.545 | -16.1 | -77.6,45.3 | 0.607 |
| | | | Control | <i>Ref</i> | | | <i>Ref</i> | | |
| | | | | | | | | | |
| | | Age | Per year | <i>NT</i> | | | -3.8 | -18.9,11.2 | 0.617 |
| | | | | | | | | | |
| | | Gender | Male | | | | 11.6 | -46.4,69.6 | 0.695 |
| | | | Female | <i>NT</i> | | | <i>Ref</i> | | |
| | | | | | | | | | |
| | | Ethnicity | | <i>NT</i> | | | -5.9 | -24.0,12.3 | 0.527 |
| | | | | | | | | | |
| | | Irregularity at baseline | Per mm | <i>NT</i> | | | 17.3 | -2.4,37.0 | 0.085 |
| | | | | | | | | | |
| | | Extraction | Yes | | | | -11.2 | -74.8,52.5 | 0.731 |
| | | | No | <i>NT</i> | | | <i>Ref</i> | | |
| | | | | | | | | | |
| | Secondary outcome: alignment rate | Factor | | b | 95% CI | P | b | 95% CI | P |
| | | BMI group | Obese | 0.022 | 0.009,0.035 | 0.001 | 0.021 | 0.008,0.033 | 0.002 |
| | | | Control | <i>Ref</i> | | | <i>Ref</i> | | |
| | | Time point* | | | | | | | |
| | | 1 week | | <i>Ref</i> | | | <i>Ref</i> | | |
| | | Completion of alignment | | -0.033 | -0.048,-0.018 | <0.001 | -0.029 | -0.046,-0.0129 | <0.001 |
| | | | | | | | | | |
| | | Age | Per year | <i>NT</i> | | | 0.001 | -0.003,0.005 | 0.614 |
| | | | | | | | | | |
| | | Gender | Male | | | | 0.003 | -0.009,0.015 | 0.625 |
| | | | Female | <i>Ref</i> | | | <i>Ref</i> | | |
| | | | | | | | | | |
| | | Ethnicity | | <i>NT</i> | | | -0.000 | -0.005,0.004 | 0.828 |
| | | | | | | | | | |
| | | Irregularity at each phase start | Per mm | <i>NT</i> | | | 0.005 | 0.000,0.010 | 0.037 |
| | | | | | | | | | |
| | | Extraction | Yes | | | | -0.001 | -0.014,0.012 | 0.910 |
| | | | No | <i>Ref</i> | | | <i>Ref</i> | | |

b, unstandardized regression coefficient; CI, confidence interval; Ref, reference.

*Interaction of obesity with time found to be non-significant (P=0.171) and was dropped from the model.

Appendix Table 4 Sensitivity analysis: regression analysis on the primary (time to completion of alignment in days) and secondary outcome (rate of tooth movement in mm/day) for the category of patients with moderate initial crowding (<7mm; n=24).

| | | Factor | | Crude model | | | Adjusted model | | |
|---|------------------------------------|----------------------------------|----------|-------------|---------------|-------|----------------|--------------|-------|
| | | | | b | 95% CI | P | b | 95% CI | P |
| | | | | | | | | | |
| Sensitivity analysis: initial crowding <7 mm (n=24) | Primary outcome: time to alignment | BMI group | Obese | -18.04 | -85.68,49.59 | 0.601 | 2.7 | -78.2,83.6 | 0.948 |
| | | | Control | <i>Ref</i> | | | <i>Ref</i> | | |
| | | | | | | | | | |
| | | Age | Per year | <i>NT</i> | | | 8.1 | -17.8,34.0 | 0.540 |
| | | | | | | | | | |
| | | Gender | Male | | | | -40.2 | -117.1,36.7 | 0.305 |
| | | | Female | <i>NT</i> | | | <i>Ref</i> | | |
| | | | | | | | | | |
| | | Ethnicity | | <i>NT</i> | | | 8.2 | -14.3,30.8 | 0.474 |
| | | | | | | | | | |
| | | Irregularity at baseline | Per mm | <i>NT</i> | | | 7.2 | -34.1,48.4 | 0.734 |
| | | | | | | | | | |
| | | Extraction | Yes | | | | -32.7 | -227.6,162.2 | 0.742 |
| | | | No | <i>NT</i> | | | <i>Ref</i> | | |
| | | | | | | | | | |
| | Secondary outcome: alignment rate | | | b | 95% CI | P | b | 95% CI | P |
| | | BMI group | Obese | 0.013 | -0.002,0.028 | 0.100 | 0.013 | 0.001,0.025 | 0.039 |
| | | | Control | <i>Ref</i> | | | <i>Ref</i> | | |
| | | Time point* | | | | | | | |
| | | 1 week | | <i>Ref</i> | | | <i>Ref</i> | | |
| | | Completion of alignment | | -0.015 | -0.028,-0.001 | 0.030 | -0.012 | -0.026,0.003 | 0.117 |
| | | | | | | | | | |
| | | Age | Per year | <i>NT</i> | | | -0.001 | -0.005,0.002 | 0.484 |
| | | | | | | | | | |
| | | Gender | Male | | | | 0.003 | -0.009,0.014 | 0.657 |
| | | | Female | <i>NT</i> | | | <i>Ref</i> | | |
| | | | | | | | | | |
| | | Ethnicity | | <i>NT</i> | | | -0.001 | -0.005,0.002 | 0.491 |
| | | | | | | | | | |
| | | Irregularity at each phase start | Per mm | <i>NT</i> | | | 0.007 | -0.001,0.015 | 0.104 |
| | | | | | | | | | |
| | | Extraction | Yes | | | | -0.005 | -0.020,0.009 | 0.477 |
| | | | No | <i>NT</i> | | | <i>Ref</i> | | |

b, unstandardized regression coefficient; CI, confidence interval; Ref, reference.

*Interaction of obesity with time found to be non-significant (P=0.171) and was dropped from the model.

Appendix Table 5 Results of regression models assessing the effect of obesity and time-variation during orthodontic treatment on the levels of clinical indices and GCF biomarkers. Explorative interactions between obesity and time were tested in each case, but were dropped from the model if they were not statistically significant.

| Biomarker transformation | Factor | | b | 95% CI | P |
|-------------------------------|-------------|-------------------------|------------|-------------|--------------|
| uWMS | Obese | Time | -0.03 | -0.13,0.08 | 0.594 |
| Log ₁₀ transformed | Time | Baseline | <i>Ref</i> | | |
| | | 1 hour | 0.19 | 0.15,0.25 | <0.001 |
| | | 1 week | 0.18 | 0.11,0.26 | <0.001 |
| | | Completion of alignment | 0.18 | 0.12,0.24 | <0.001 |
| | Interaction | | | | 0.502 |
| | | | | | |
| Plaque index | Obese | | 0.03 | -0.05,0.12 | 0.460 |
| Log ₁₀ transformed | Time | Baseline | <i>Ref</i> | | |
| | | 1 hour | - | | |
| | | 1 week | 0.16 | 0.07,0.26 | 0.001 |
| | | Completion of alignment | 0.34 | 0.27,0.41 | <0.001 |
| | Interaction | | | | 0.353 |
| | | | | | |
| Gingival index | Obese | | 0.04 | -0.05,0.12 | 0.391 |
| Log ₁₀ transformed | Time | Baseline | <i>Ref</i> | | |
| | | 1 hour | - | | |
| | | 1 week | 0.14 | 0.06,0.21 | 0.001 |
| | | Completion of alignment | 0.30 | 0.24,0.36 | <0.001 |
| | Interaction | | | | 0.206 |
| | | | | | |
| GCF flow-rate | Obese | | 0.08 | 0.04,0.13 | <0.001 |
| Log ₁₀ transformed | Time | Baseline | <i>Ref</i> | | |
| | | 1 hour | 0.10 | 0.05,0.15 | <0.001 |
| | | 1 week | 0.10 | 0.06,0.14 | <0.001 |
| | | Completion of alignment | 0.09 | 0.04,0.13 | <0.001 |
| | Interaction | | | | 0.282 |
| | | | | | |
| Adiponectin | Obese | | 0.08 | -0.12,0.28 | 0.417 |
| Log ₁₀ transformed | Time | Baseline | <i>Ref</i> | | |
| | | 1 hour | 0.15 | 0.04,0.26 | 0.010 |
| | | 1 week | 0.23 | 0.10,0.36 | <0.001 |
| | | Completion of alignment | 0.15 | 0.01,0.28 | 0.031 |
| | Interaction | | -0.07 | -0.13,-0.01 | 0.027 |
| | | | | | |
| Leptin | Obese | | 6.41 | 1.71,11.10 | 0.007 |
| Square-root transformed | Time | Baseline | <i>Ref</i> | | |
| | | 1 hour | -2.44 | -5.18,0.29 | 0.080 |
| | | 1 week | -1.21 | -1.85,4.27 | 0.439 |
| | | Completion of alignment | -4.44 | -8.50,-0.38 | 0.032 |
| | Interaction | | | | 0.556 |
| | | | | | |
| Resistin | Obese | | 0.60 | 0.44,0.76 | <0.001 |
| Log ₁₀ transformed | Time | Baseline | <i>Ref</i> | | |
| | | 1 hour | 0.13 | -0.04,0.30 | 0.124 |
| | | 1 week | 0.03 | -0.16,0.21 | 0.757 |

| | | | | | |
|-------------------------------|-------------|-------------------------|------------|-------------|--------------|
| | | Completion of alignment | -0.05 | -0.18,0.09 | 0.519 |
| | Interaction | | | | 0.975 |
| | | | | | |
| MPO | Obese | | 1.36 | 1.11,1.62 | <0.001 |
| Log ₁₀ transformed | Time | Baseline | <i>Ref</i> | | |
| | | 1 hour | 0.36 | 0.13,0.59 | 0.003 |
| | | 1 week | 0.56 | 0.39,0.74 | <0.001 |
| | | Completion of alignment | 0.69 | 0.52,0.87 | <0.001 |
| | Interaction | | | | 0.994 |
| | | | | | |
| CRP | Obese | | -0.02 | -0.24,0.20 | 0.860 |
| Log ₁₀ transformed | Time | Baseline | <i>Ref</i> | | |
| | | 1 hour | 0.10 | -0.04,0.23 | 0.149 |
| | | 1 week | 0.27 | 0.09,0.46 | 0.004 |
| | | Completion of alignment | 0.26 | 0.07,0.44 | 0.006 |
| | Interaction | | | | 0.192 |
| | | | | | |
| MMP8 | Obese | | 0.62 | 0.32,0.93 | <0.001 |
| Log ₁₀ transformed | Time | Baseline | <i>Ref</i> | | |
| | | 1 hour | 0.16 | 0.05,0.28 | 0.007 |
| | | 1 week | 0.12 | 0.01,0.23 | 0.037 |
| | | Completion of alignment | 0.05 | -0.07,0.17 | 0.446 |
| | Interaction | | | | 0.995 |
| | | | | | |
| MMP9 | Obese | | 0.06 | -0.02,0.15 | 0.149 |
| Log ₁₀ transformed | Time | Baseline | <i>Ref</i> | | |
| | | 1 hour | 0.38 | 0.28,0.49 | <0.001 |
| | | 1 week | 0.35 | 0.25,0.44 | <0.001 |
| | | Completion of alignment | 0.08 | -0.03,0.19 | 0.181 |
| | Interaction | | | | 0.388 |
| | | | | | |
| TIMP1 | Obese | | 0.61 | 0.38,0.84 | <0.001 |
| Log ₁₀ transformed | Time | Baseline | <i>Ref</i> | | |
| | | 1 hour | -0.04 | -0.20,0.13 | 0.668 |
| | | 1 week | 0.25 | 0.07,0.42 | 0.005 |
| | | Completion of alignment | 0.42 | 0.23,0.60 | <0.001 |
| | Interaction | | -0.08 | -0.15,-0.00 | 0.040 |
| | | | | | |
| MMP8/TIMP1 | Obese | | 0.20 | -0.14,0.54 | 0.254 |
| Log ₁₀ transformed | Time | Baseline | <i>Ref</i> | | |
| | | 1 hour | 0.24 | 0.03,0.45 | 0.027 |
| | | 1 week | -0.05 | -0.22,0.13 | 0.600 |
| | | Completion of alignment | -0.25 | -0.42,-0.08 | 0.003 |
| | Interaction | | | | 0.166 |
| | | | | | |
| MMP9/TIMP1 | Obese | | -0.34 | -0.57,-0.11 | 0.004 |
| Log ₁₀ transformed | | | | | |
| | Time | Baseline | <i>Ref</i> | | |
| | | 1 hour | 0.46 | 0.33,0.59 | <0.001 |
| | | 1 week | 0.18 | 0.02,0.34 | 0.024 |
| | | Completion of alignment | -0.23 | -0.35,-0.10 | <0.001 |

| | | | | | |
|-------------------------------|-------------|-------------------------|------------|------------|--------|
| | Interaction | | | | 0.239 |
| | | | | | |
| RANKL | Obese | | 0.38 | 0.28,0.48 | <0.001 |
| Log ₁₀ transformed | Time | Baseline | <i>Ref</i> | | |
| | | 1 hour | 0.01 | -0.06,0.09 | 0.733 |
| | | 1 week | 0.03 | -0.06,0.12 | 0.532 |
| | | Completion of alignment | 0.37 | 0.27,0.47 | <0.001 |
| | Interaction | | | | 0.089 |

b, unstandardized regression coefficient; CI, confidence interval; Ref, reference; GCF, gingival crevicular fluid; MPO, myeloperoxidase; CRP, C reactive protein; MMP8, matrix metalloproteinase-8; MMP9, matrix metalloproteinase-9; TIMP1, tissue inhibitor of metalloproteinase 1; RANKL, receptor activator of nuclear factor kappa-B ligand.

Appendix Table 6 Results of regression models assessing the association of time-variation during orthodontic treatment, baseline irregularity, and biomarker levels with tooth alignment rate in mm/day

| Biomarker transformation | Factor | b | 95% CI | P |
|-------------------------------|-----------------------|--------|---------------|--------|
| Leptin | Time | -0.017 | -0.029,-0.005 | 0.005 |
| Square-root transformed | Baseline irregularity | 0.007 | 0.005,0.010 | <0.001 |
| | Leptin | 0.001 | 0.000,0.001 | 0.006 |
| | | | | |
| Resistin | Time | -0.020 | -0.030,-0.010 | <0.001 |
| Log ₁₀ transformed | Baseline irregularity | 0.007 | 0.005,0.010 | <0.001 |
| | Resistin | 0.013 | 0.006,0.020 | 0.001 |
| | | | | |
| MPO | Time | -0.022 | -0.032,-0.012 | <0.001 |
| Log ₁₀ transformed | Baseline irregularity | 0.007 | 0.005,0.009 | <0.001 |
| | MPO | 0.010 | 0.004,0.014 | <0.001 |
| | | | | |
| MMP8 | Time | -0.021 | -0.031,-0.010 | <0.001 |
| Log ₁₀ transformed | Baseline irregularity | 0.007 | 0.005,0.010 | <0.001 |
| | MMP8 | 0.003 | -0.004,0.010 | 0.415 |
| | | | | |
| TIMP1 | Time | -0.022 | -0.032,-0.011 | <0.001 |
| Log ₁₀ transformed | Baseline irregularity | 0.007 | 0.005,0.010 | <0.001 |
| | TIMP1 | 0.004 | -0.004,0.012 | 0.335 |
| | | | | |
| MMP9/TIMP1 | Time | -0.022 | -0.033,-0.010 | <0.001 |
| Log ₁₀ transformed | Baseline irregularity | 0.007 | 0.005,0.009 | <0.001 |
| | MMP9/TIMP1 | -0.003 | -0.012,0.007 | 0.592 |
| | | | | |
| RANKL | Time | -0.025 | -0.035,-0.014 | <0.001 |
| Log ₁₀ transformed | Baseline irregularity | 0.007 | 0.005,0.010 | <0.001 |
| | RANKL | 0.011 | 0.000,0.022 | 0.040 |

MPO, myeloperoxidase; MMP8, matrix metalloproteinase-8; TIMP1, tissue inhibitor of metalloproteinase 1; RANKL, receptor activator of nuclear factor kappa-B ligand.